

Cell line designation: IPLB-HvE6a

Tissue source: *Heliothis virescens* embryos

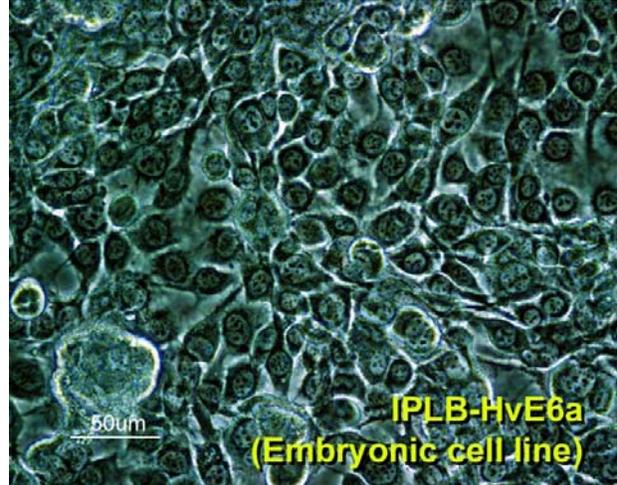
Date initiated: 15 June 1995

Morphology: fibroblastoid, strongly attached cells

Culture medium: Modified TC100 (=BML-TC/10, Gardiner and Stockdale, J. Invertebr. Pathol. 25: 363-370, 1975, modified as follows):

Complete Medium

TC100	100 ml
Fetal Bovine Serum	10 ml
stock "P"	5.0 ml
vitamin stock	0.2 ml
trace minerals	0.1 ml
iron soln.	0.067 ml
Glycerol (50% in water)	0.32 ml
Glutamine (2 g/l)	2.0 ml



(see below for stock solutions)

Subculture procedure: At one week intervals, a confluent culture is split by trypsinization. Remove the medium from the culture and rinse the cells with buffer (see below)(use about 2 ml buffer to rinse a 25-cm² flask). The rinse is discarded and 1.0 ml trypsin (0.05 mg/ml VMF Trypsin, Worthington Biochem. cat. # LS04454 in buffer) is added. The flask is tilted to be sure entire monolayer is coated with solution then all but 0.3 ml is removed and discarded. Wait 5-10 min. Tap the culture on the bench top and look to see if some cells have detached. When they have, add 4.0 ml fresh medium and suspend cells by repeated pipetting over the flask surface. Transfer 0.7 ml cell suspension into 3.3 ml medium in a new flask (=~1:6 split). Cells are maintained at 26°C.

Buffer for trypsinization

NaCl	800 mg
KH ₂ PO ₄	20 mg
KCl	20 mg
Na ₂ HPO ₄ ×7H ₂ O	150 mg
Na ₂ EDTA	23 mg

in water to 100 ml

Adjust osmotic pressure with 15% NaCl to 350 mOsm/kg and pH to 7.0 with 2N NaOH. Filter sterilized through 0.2 µm filter and store at 4°C.

Virus susceptibility: Cells are susceptible (with complete occlusion body formation) to *Autographa californica* nucleopolyhedrovirus, *Anagrapha falcifera* nucleopolyhedrovirus, *Anticarsa gemmatalis* nucleopolyhedrovirus, *Rachoplusia ou* nucleopolyhedrovirus, *Orgyia pseudotsugata* nucleopolyhedrovirus, and *Helicoverpa zea* nucleopolyhedrovirus.

Comments: This is an attached strain isolated from IPLB-HvE6

Reference: Lynn, D. E. and Shapiro, M. (1998) New cell lines from *Heliothis virescens*: Characterization and Susceptibility to Baculoviruses. J. Invertebr. Pathol.72:276-280.

MEDIA STOCK SOLUTIONS

Stock "P"

HyPep Dev 4601 (Quest International)	5 g
Primatone RL (Sheffield Products)	5 g
TC grade water	100 ml

Autoclave, store 4°C

Iron Soln.

FeSO ₄ .7H ₂ O	0.0823 g
Aspartic acid	0.0532 g
TC grade water	100 ml

Filter 0.2 um, store 4°C

Vitamin Stock

Thiamine-HCl	4.8 mg
Riboflavin	4.8 mg
Calcium Pantothenate	4.8 mg
Pyridoxine HCl	12. mg
Para-amino benzoic acid	9.6 mg
Niacin	4.8 mg
Biotin	4.8 mg
TC Grade water	60 ml

Filter 0.2 um, store 4°C

Trace Minerals

ZnCl ₂	4.0 mg
MnCl ₂ .4H ₂ O	2.0 mg
CuCl ₂ .2H ₂ O	19.5 mg
(NH ₄) Mo ₇ O ₂₄ .4H ₂ O	4.0 mg
CoCl ₂ .6H ₂ O	5.0 mg
TC Grade Water	100 ml

Filter 0.2 um, store 4°C